METHOD FOR RECOVERING THE FROZEN AND THAWED FUNGAL CULTURES (CRYOTUBE) / OPENING OF AMPULES AND REVIVAL OF L-DRIED SPECIMENS

After accept this shipped specimens, please start recovering or culturing the specimens by following the appropriate procedure (for the specimens in test tubes, cryotubes, or ampules) described on this paper.

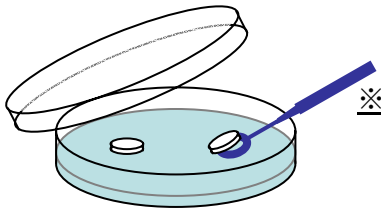
If you accept the specimens in **test tubes**, please start culturing as soon as possible by transferring the specimens to the flesh medium which is described on the enclosed document.

If you are unable to recover the specimen by the following the procedure described on this paper, please inform us as soon as possible. We will accept claims concerning the shipped specimens (in test tubes, cryotubes and ampules) within one month after your receipt.

METHOD FOR RECOVERING THE FROZEN AND THAWED FUNGAL CULTURES IN CRYOTUBE

The two disks contained in the cryotube provided here were bored out of an agar plate together with the fungal colony growing on it. They were stored frozen and thawed just before delivery. To recover the specimen, please follow the procedure described below.

1. Prepare the growth medium noted on a separate sheet.
2. Disinfect the surface of the cryotube with a piece of 75% alcohol-dampened gauze.
3. Transfer aseptically the agar disk from the cryotube to the medium with an inoculation loop.



※ Please pay attention not to crush the disks upon transfer.

4. Incubate the disk under the conditions noted on a separate sheet. For slow growing species, continue incubation for 2 weeks or more.

If you do not wish to recover this specimen (in cryotubes) immediately after receipt, you may keep the cryotubes in a refrigerator. However, we recommend its recovery within a few days.

OPENING OF AMPULES AND REVIVAL OF L-DRIED SPECIMENS (AMPULES)

L-dried cells are supplied in a vacuum-sealed glass. To open and revive the L-dried specimens, please refer to the procedure as described below and also to the backpage illustration.

1. Prepare rehydration fluid and growth medium specified.
2. Score the ampule near the middle point of the cotton plug with an ampule cutter* **(Figure 1)**.
3. Disinfect the ampule with alcohol-dampened gauze **(Figure 2)**.
4. Wrap sterile gauze around the ampule and break it carefully (do not use the alcohol-dampened gauze) **(Figures 3 and 4)**.
5. Immediately add about 0.2 ml of rehydration fluid to the L-dried cells with a sterile Pasteur pipet **(Figure 5)**.
   Mix well and transfer the cell suspension to a growth medium (broth, slant, plate) **(Figure 6)**.

[The reactivation procedure for a bacteriophage]

This step is the same as described above (steps 1-5). After step 5, soft-agar (0.6 %) containing host bacteria is to be overlaid on a plain agar plate and the rehydration fluid containing the bacteriophage be spread onto the solidified soft-agar. Leave a small portion of the plate without the bacteriophage so that bacterial lawn with and without bacteriophage may be compared. Appropriate host bacteria are needed to reactivate bacteriophages. If you do not have a suitable host strain, you need to order the host simultaneously.

(Continue to the next page)

6. Incubate under the conditions specified for the cells.
Some cells may exhibit a prolonged lag period before growth. If such a case occurs, continue the incubation for about 2 weeks at an appropriate temperature before discarding as inviable.

7. All the remains of the ampule should be sterilized before discarding.

If you do not wish to recover this specimen (in ampules) immediately after receipt, you may keep the ampules in a refrigerator.

INSTRUCTIONS FOR OPENING OF AMPULES AND REVIVAL OF L-DRIED SPECIMENS

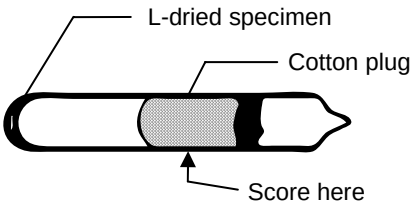
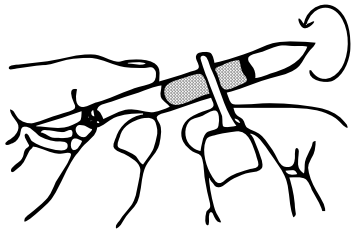
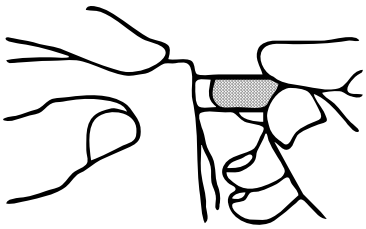


Figure. 1



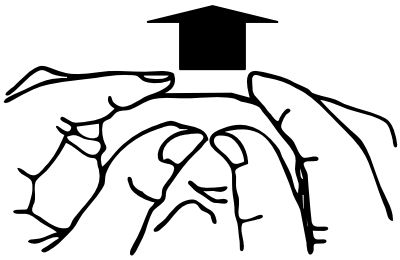
Score the glass wall with an ampule cutter* or a file.

Figure. 2



Disinfect the ampule with alcohol-dampened gauze.

Figure. 3



Wrap sterile gauze (do not use an alcohol-dampened gauze) and break it by applying pressure to the side opposite the score.

Figure. 4

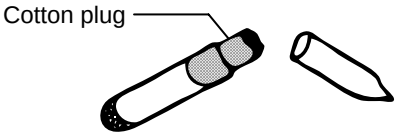
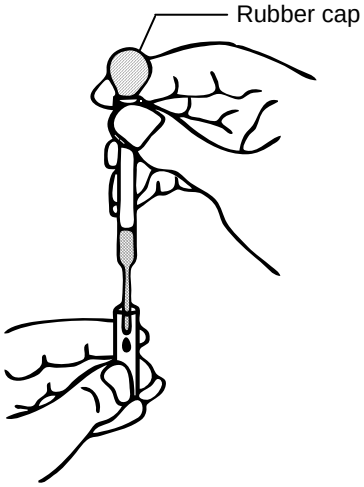
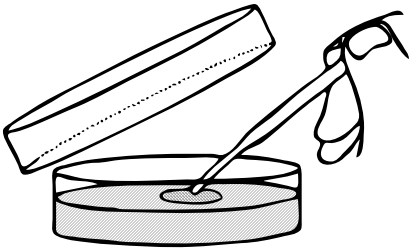


Figure. 5



Remove the cotton plug, add re-hydration fluid with a sterile Pasteur pipette and mix gently.

Figure. 6



Transfer the cell suspension to a recommended growth medium (for bacteria and archaea, transfer the suspension also into a liquid medium).

*If you need additional information about the ampule cutter, please consult any of the companies listed below.

Tozai Tsusho Co., Ltd.
Queen's bldg, 2-9-7, Nishi shinbashi, Minato-ku,
Tokyo 105-0003, Japan
TEL +81-3-3502-8231 FAX +81-3-3502-8234

Iwata Glass Kogyo Co., Ltd
4-11, Mukojima-cho Kadoma-city, Osaka 571-0051,
Japan
TEL +81-6-6902-9391 FAX +81-6-6902-0330

Biological Resource Center (NBRC),
National Institute of Technology and Evaluation
2-5-8, Kazusakamatari, Kisarazu, Chiba, 292-0818 Japan
Tel: +81-438-20-5763, Fax: +81-438-52-2329

NBRC
NITE Biological Resource Center